

## REMARKS

### Status of the claims

Claims 1-18 are pending in the application. Claims 7-9 and 16-18 are withdrawn from consideration. Claims 1-6 and 10-15 are rejected.

Claims 1 and 10 are currently amended. Claims 7-9 and 16-18 are withdrawn. No new matter is added herein.

### Claim Amendment

Claims 1 and 10 are amended to overcome the 35 U.S.C. §112, first paragraph rejections. Amended claim 1 is drawn to a method of treating an individual having irritable bowel syndrome. Such a method comprises the step of administering to the individual a pharmacologically effective dose of a lumenally active anti-inflammatory compound with minimal or no systemic side effects. This amendment is supported by description of pg 16, lines 3-10 of the instant invention.

Amended claim 10 is drawn to a method of reducing the intensity of symptoms of irritable bowel syndrome in an individual in need of such treatment. Such a method comprises the step of administering to the individual a pharmacologically effective dose of a lumenally active anti-inflammatory compound with minimal or no systemic side effects such that the administration increases the threshold of pain to colorectal distention, thereby alleviating the symptoms of irritable bowel syndrome in the individual. This amendment is

supported by description on pg 16, lines 3-10 and Example of the instant invention.

35 U.S.C. §112, First Paragraph rejection

Claims 10-16 are rejected under 35 U.S.C. §112, first paragraph for lack of enablement. Applicants respectfully traverse this rejection.

In general, the Examiner states that the instant specification while being enabled for the “treatment of irritable bowel syndrome....with a pharmacologically effective amount” does not provide enablement for the “inhibiting the onset of symptoms of irritable bowel syndrome....with a prophylactically effective amount”. Based on this, the Examiner states that the specification does not enable any person skilled in the art to which it pertains or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

The following are the specific reasons cited by the Examiner for this rejection: First, the invention encompasses the actual inhibition of irritable bowel syndrome or a related disorder such that the subject does not contract irritable bowel syndrome or any related disorder. Second, the claims encompass inhibition of a complex irritable bowel syndrome or a related disorder in humans which has no anatomic cause. Third, the instant specification lacks guidance towards inhibition of the onset of symptoms of irritable bowel syndrome or any related disorder with prophylactic dose of an anti-inflammatory compound. Fourth, the working example in the instant specification is directed towards

treatment of irritable bowel syndrome with effective amounts rather than inhibition with prophylactic dose of anti-inflammatory compound. Fifth, the prior art is deficient in the teachings of whether administration of the compound similar to the one claimed herein inhibits development of irritable bowel syndrome and related disorder. Sixth, the lack of significant guidance from the specification or prior art with regard to the actual inhibition of irritable bowel syndrome with prophylactic dose of the claimed compounds makes practicing the claimed invention unpredictable. Seventh, one skilled in the art would have to perform undue experimentation to practice the claimed invention since the specification is deficient with regards to appropriate carrier, compound dosage, duration of treatment, route of administration, etc to inhibit the onset of symptoms of irritable bowel syndrome and related disorder.

Claim 10 is amended as discussed supra. Based on this amendment, the claim is no longer drawn to inhibiting the onset of symptoms of irritable bowel syndrome or a related disorder but is drawn to a method of alleviating the symptoms of irritable bowel syndrome in an individual. The instant specification discloses that lumenally active anti-inflammatory or immunosuppressive compound with minimal or no systemic side effects can be administered orally to treat irritable bowel syndrome and related disorders. These compounds share the same features i.e. they are active in the lumen of the GI tract but do not exert systemic effects because of poor absorption or rapid metabolism by the liver before they reach the systemic circulation. Examples of these compounds include but are not limited to beclomethasone or budesonide (pg. 16, lines 1-15).

The instant specification further teaches that although this syndrome has traditionally been considered a “functional” gastrointestinal disorder, there is increasing evidence to implicate a causative role for subtle inflammation in the form of infiltrating lymphocytes, mast cells and other immune-competent cells. Although anti-inflammatory or immunosuppressive therapy is a logical approach to the treatment of these patients, traditional forms of these therapies are associated with significant systemic toxicity which makes their use difficult to justify in the treatment of a benign syndrome (pg. 16, line 18- pg. 17, line 16).

Thus, the novelty of the instant invention lies, at least in part, in the fact that it demonstrates that anti-inflammatory agents can be administered orally to treat irritable bowel syndrome and such administration is associated with minimal or no systemic side effects. The instant invention examined the effect of oral beclomethasone dipropionate on acetic acid sensitized rats. Since this condition is characterized by abdominal pain associated with a change in bowel movements, the behavioral response of the rats subjected to colorectal distention with regards to abdominal discomfort was graded. Sensitized rats that were fed beclomethasone dipropionate showed higher pain threshold to graded colorectal distention compared to the vehicle treated group. In other words, oral administration of anti-inflammatory compound such as beclomethasone dipropionate alleviated abdominal pain associated with irritable bowel syndrome.

Applicants would like to respectfully point out that “the specification need not contain an example if the invention is otherwise disclosed in such a manner that one skilled in the art will be able to practice it without undue

experimentation. Lack of a working example, however, is a factor to be considered, especially in a case involving an unpredictable and undeveloped art. But because an enabling disclosure is required, Applicants need not describe all actual embodiments” (M.P.E.P. 2164.02). Applicants contend that the results disclosed in the instant specification are sufficient to enable the instantly claimed methods in irritable bowel syndrome as claimed. Therefore, a fair reading of the instant specification should enable one of skill in the art to make and use the claimed invention without undue experimentation. Accordingly, based on the above-discussed amendment and remarks, Applicants respectfully request the withdrawal of rejection of claims 10-16 under 35 U.S.C. §112, first paragraph.

Claims 1-6 and 10-15 are rejected under 35 U.S.C. §112, first paragraph, for failing to comply with the written description requirement. Applicants respectfully traverse this rejection.

The Examiner states that the claims by reciting “a related disorder” encompasses a broad genus of a related disorder of irritable bowel disease. More specifically, the Examiner states that the instant specification does not describe or exemplify all related disorder, much less any symptoms and signs, lab findings, anatomical cause, demographic data of such related disorder qualified as a related disorder of irritable bowel syndrome. Accordingly, the Examiner states that the instant specification does not provide a basis for one of skill in the art to envision the related disorders of irritable bowel syndrome. Given this lack of description of a sufficient number of diseases of the representative species encompassed by the

genus of the claim, the Examiner states that the instant specification fails to describe the claimed invention in such full, clear, concise and exact terms regarding the related disorders of irritable bowel syndrome that a skilled artisan would not recognize that Applicants were in possession of the claimed invention "a related disorder".

The recitation of " a related disorder" in claims 1 and 10 is deleted. As discussed supra, the teachings of the instant invention thoroughly support the inventions claimed in instant claims 1 and 10. Hence, amended claims 1 and 10 satisfy the written description requirement. Accordingly, based on the claim amendments and above-mentioned remarks, Applicants respectfully request the withdrawal of rejection of claims 1-5 and 10-14 under 35 U.S.C. §112, first paragraph.

#### 35 U.S.C. §102(b) rejection

Claims 1-5 and 10-14 are rejected under 35 U.S.C. §102(b) as being anticipated by **Chiesi et al** (WO 00/06132A2) as evidenced by **Basu et al** (U.S. 2002/0025348A1). Applicants respectfully traverse this rejection.

The Examiner states that **Chiesi et al** teach a pharmaceutical formulation for the treatment of irritable bowel disease that contains beclomethasone dipropionate (BDP) as the active ingredient (abstract). **Chiesi et al** teach that the formulation demonstrate no systemic absorption of beclomethasone dipropionate and its major active metabolites (pg. 14, Example 5). **Chiesi et al** also

teach the amount of beclomethasone dipropionate to be employed includes 3mg and 5mg (pg. 3, lines 9-11 and pg. 8-9, Examples 1 and 2).

The Examiner further states that **Basu et al** report that the inflammatory bowel disease (IBD) and irritable bowel disease are related (pg. 1, 0003). **Basu et al** report that irritable bowel syndrome also tends to occur in inflammatory bowel disease patients who are in remission from their inflammatory bowel disease symptomologies (pg. 1, 0009). Accordingly, the Examiner states that **Chiesi et al** teach that the same individual having a related disorder of irritable bowel syndrome, inflammatory bowel disease as evidenced by **Basu et al** treated with the same active agent comprising the same effective dosages providing no systemic absorption of beclomethasone dipropionate as instantly claimed by the Applicant. Further, the mechanism of providing lumenally active anti-inflammatory action of beclomethasone dipropionate by which beclomethasone dipropionate gives the pharmacological effect of treating same disease condition does not alter the fact that the compound has been previously used to obtain the same pharmacological effects which would result from the claimed method. The patient, condition to be treated and the effect are the same.

With regard to claim 10, the Examiner states that **Basu et al** report that irritable bowel syndrome tends to occur in inflammatory bowel disease patients who are in remission from their symptomologies. Therefore, the inflammatory bowel disease patients disclosed by **Chiesi et al** are the patients in need of treatment of irritable bowel syndrome because irritable bowel syndrome tends to occur in inflammatory bowel disease patients as taught by **Basu et al**. Therefore, claim 10

drawn to inhibiting the onset of symptoms of irritable bowel syndrome would be inherent in **Chiesi's** method of treating inflammatory bowel disease comprising identical patient who is in need of treating irritable bowel syndrome as evidenced by **Basu et al.** Applicants respectfully disagree.

The instant invention is drawn to providing a new treatment for irritable bowel syndrome (claims 1-6) or to alleviating the symptoms of irritable bowel syndrome (claims 10-15). As discussed herein, the precise pathophysiology of irritable bowel syndrome is not well understood. Irritable bowel syndrome is considered functional disorders since it lacks structural or organic changes (pg. 16, line 18-pg. 17, line 2). Hence, irritable bowel syndrome has never been considered as an inflammatory disorder.

Even if inflammation may exist in irritable bowel syndrome, it is different than a typical inflammatory bowel disease such as ulcerative colitis or Crohn's disease for two reasons. First, there is no evidence of tissue injury or destruction either at the macroscopic or microscopic level. Second, the major cell types that appear to be affected in irritable bowel syndrome are the muscle and nerves as compared to inflammatory bowel disease, where the epithelium is prominent and a major target. Therefore, even if immunocompetent cells are contributing to the pathogenesis of irritable bowel syndrome, they might be doing so by means that are not intuitively obvious and may involve different mechanisms than those in inflammatory bowel disease (pg. 13, line 15-pg. 14, line 5). Thus, the treatments claimed in the instant invention are novel since they involve the use of



an anti-inflammatory compound in the treatment of a disorder which is not considered an inflammatory disorder.

In contrast, **Chiesi et al** teach the use of topically active corticosteroid such as beclomethasone dipropionate in the treatment of inflammatory bowel diseases such as Ulcerative colitis and Crohn's disease (pg. 1, lines 7-11). In this regard, **Chiesi et al** disclose rectal or oral administration of beclomethasone dipropionate (pg. 3, lines 8-10; pg. 6, lines 11-14). There is no teaching in **Chiesi et al** that the administration of beclomethasone dipropionate is effective in treating irritable bowel syndrome.

**Basu et al** provide novel formulations of plants and extracts thereof useful in the treatment of bowel disorders. In this regard, **Basu et al** teach that the formulations of their invention can be used to treat inflammatory bowel disease and related conditions such as irritable bowel syndrome and other inflammatory disorders such as arthritis. There is no teaching in **Basu et al** of gut inflammation in irritable bowel syndrome. In fact, **Basu et al** teach that irritable bowel syndrome, unlike inflammatory bowel disease, does not have characteristic histopathological changes but rather is a functional disorder of disturbed gut motility and/or abdominal pain perception. Further, **Basu et al** teach that irritable bowel syndrome tends to occur in patients who are in remission from their inflammatory bowel disease symptomologies. This further indicates that irritable bowel syndrome and inflammatory bowel disease may not share the pathophysiology. Thus, overall, the prior art references combined do not teach that anti-inflammatory compounds

useful in treating inflammatory bowel disease will be useful in treating irritable bowel syndrome.

In order to anticipate a claim, each and every element of the claim should be described in a single prior art reference. More importantly, the identical invention must be shown in as complete detail as is contained in the instant invention. As discussed supra, whether the beclomethasone dipropionate used by **Chiesi et al** to treat inflammatory bowel disease will be useful in treating irritable bowel syndrome or alleviating the symptoms of irritable bowel syndrome cannot be anticipated based on the teaching of **Basu et al**. Accordingly, based on the above-mentioned claim amendments and remarks, Applicants respectfully request the withdrawal of rejection of claims 1-5 and 10-14 under 35 U.S.C. §102 (b).

#### 35 U.S.C. §103, Obviousness rejection

Claims 6 and 15 are rejected under 35 U.S.C. §103(a) as being unpatentable over **Chiesi et al** (WO 00/06132A2). Applicants respectfully traverse this rejection.

The Examiner states that **Chiesi et al** do not teach the specified amounts of beclomethasone as set forth in claims 6 and 15. However, the Examiner states that it would have been obvious to one of ordinary skill in the art that the amount employed by **Chiesi et al** (3mg or 5 mg) for the treatment of inflammatory bowel disease (irritable bowel syndrome related disorder) is within the recited amounts set forth in claims 6 and 15 because **Chiesi et al** teach same individual having same related disorder of irritable bowel syndrome comprising

same effective dosages providing no systemic absorption of BDP as instantly claimed by the Applicant. Therefore, the Examiner states that **Chiesi et al** obviously administered the same effective amounts within the Applicant's recited amount in order to have the same effect of treating inflammatory bowel disease which is related to irritable bowel syndrome. Additionally, the Examiner states that 5mg amount employed by **Chiesi et al** is within the mg/kg recited in claims 6 and 15 when subject to be treated weighs 50kg. For these reasons, the Examiner states that the claimed subject matter is deemed to fail to patentably distinguish over the state of the art as represented by the cited reference. Hence, the Examiner rejects the claims as being obvious. Applicant respectfully disagrees with the Examiner.

Claims 6 and 15 depend from claims 1 and 10, respectively. The teachings of the instant invention are as discussed supra. Based on these teachings, it is very clear that irritable bowel syndrome and Inflammatory bowel disease are distinct. In fact, art at the time the invention was filed and even today, teach that irritable bowel syndrome and inflammatory bowel disease are distinct clinical syndromes in variety of aspects including treatment (Bradesi et al., 2003). As discussed supra, inflammatory bowel disease and irritable bowel syndrome represent two conditions characterized by chronically recurring symptoms of abdominal pain, discomfort (urgency and bloating) and alterations in bowel habits. However, whereas inflammatory bowel disease is characterized by inflammation or ulcerations in the small and/or large intestine, such "organic" changes have traditionally not been associated with irritable bowel syndrome.

Although inflammatory bowel disease is usually classified as ulcerative colitis or Crohn disease, it also includes forms of microscopic colitis, e.g. histologic evidence of mucosal inflammation without macroscopic abnormalities. Inflammatory bowel disease is characterized by a constellation of patient-reported history and endoscopic, histopathologic and radiologic findings often with serologic correlates. Classic signs that reflect the inflammatory process within the gastrointestinal tract are rectal bleeding, diarrhea, fever and weight loss occasionally associated with extraintestinal manifestations. Interestingly, in the absence of complications, abdominal pain is not necessarily the most prominent symptom in inflammatory bowel disease despite extensive mucosal inflammation and presumably sensitization of peripheral visceral pain pathways. Genetic predisposition, environmental factors, infectious agents altered gut epithelial permeability and impaired immune responses have been incriminated in the still unclear cause of inflammatory bowel disease.

In contrast, irritable bowel syndrome is classified as functional bowel disorder and is currently diagnosed on the basis of a characteristic cluster of symptoms in the absence of detectable structural abnormalities. As a matter of fact, according to the currently used symptom criteria (Rome criteria), once organic changes are detected, a diagnosis of functional disorder can no longer be made. Due to the non-specificity of the cardinal symptoms of abdominal pain or abdominal discomfort, the current diagnosis of irritable bowel syndrome applies to a heterogeneous group of patients, even after attempts to define subgroups based on predominant bowel habit. Current theories to explain the

pathophysiology of irritable bowel syndrome include alteration in the visceral perception, gastrointestinal motility and gut epithelia and immune function. Considerable evidence supports the role of psychosocial and physical stressors as central and peripheral triggers respectively of first symptom onset or exacerbation. Although there is a considerable interest in the putative role of low-grade chronic inflammation in the pathogenesis of irritable bowel syndrome, enhanced responsiveness to psychosocial and physical stressors has been suggested as a plausible mechanism that could explain most clinical and experimental findings in irritable bowel syndrome. Thus, there is ample evidence to show that irritable bowel syndrome and inflammatory disease are distinct diseases.

**Chiesi et al** teach the use of topically active corticosteroid such as beclomethasone dipropionate in the treatment of inflammatory bowel diseases such as Ulcerative colitis and Crohn's disease (pg. 1, lines 7-11). In this regard, **Chiesi et al** disclose rectal or oral administration of beclomethasone dipropionate (pg. 3, lines 8-10; pg. 6, lines 11-14). There is no teaching or suggestion in **Chiesi et al** that the administration of beclomethasone dipropionate is effective in treating irritable bowel syndrome. Additionally, one skilled in the art would not be motivated to use beclomethasone dipropionate to treat irritable bowel syndrome or alleviate its symptoms for the following reasons. First, the information in the art at the time of filing of the instant invention which teaches that irritable bowel syndrome and inflammatory disorder are distinct. Second, lack of histological evidence of inflammation in irritable bowel syndrome (pg. 13, line 15-pg. 14, line 5). Thus,

independent claims 1 and 10 and their dependent claims 6 and 15, respectively are not prima facie obvious over **Chiesi** et al. accordingly, based on the claim amendments and above-mentioned remarks, Applicants respectfully request the withdrawal of rejection of claims 6 and 15 under 35 U.S.C. §103.

This is intended to be a complete response to the Office Action mailed March 23, 2007. If any issues remain outstanding, please telephone the undersigned attorney of record for immediate resolution.

Respectfully submitted,

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# Inflammatory bowel disease and irritable bowel syndrome: separate or unified?

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Both irritable bowel syndrome and inflammatory bowel diseases share symptoms of altered bowel habits associated with abdominal pain or discomfort. Irritable bowel syndrome has been referred to as a functional bowel disorder, which is diagnosed by a characteristic cluster of symptoms in the absence of detectable structural abnormalities. Inflammatory bowel disease is a heterogeneous group of disorders characterized by various forms of chronic mucosal and/or transmural inflammation of the intestine. In this review, the authors discuss recent evidence suggesting several potential mechanisms that might play a pathophysiologic role in both syndromes. Possible shared pathophysiologic mechanisms include altered mucosal permeability, an altered interaction of luminal flora with the mucosal immune system, persistent mucosal immune activation, alterations in gut motility, and a role of severe, sustained life stressors in symptom modulation. It is proposed that similarities and differences between the two syndromes can best be addressed within the framework of interactions between the central nervous system and the gut immune system. Based on recent reports of low-grade mucosal inflammation in subpopulations of patients meeting current diagnostic criteria for irritable bowel syndrome, therapeutic approaches shown to be effective in inflammatory bowel disease, such as probiotics, antibiotics, and antiinflammatory agents, have been suggested as possible therapies for certain patients with irritable bowel syndrome.

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## Abbreviations

HPA	hypothalamic-pituitary-adrenal
IBD	inflammatory bowel diseases
IBS	irritable bowel syndrome
IELs	intraepithelial lymphocytes
INOS	inducible nitric oxide synthase
MAPK	mitogen-activated protein kinase
PI-IBS	postinfectious irritable bowel syndrome
Th1	T helper 1
TNF- $\alpha$	tumor necrosis factor- $\alpha$

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Inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) represent two conditions characterized by chronically recurring symptoms of abdominal pain, discomfort (urgency and bloating) and alterations in bowel habits. However, whereas IBD is characterized by inflammation or ulcerations in the small and/or large intestine, such "organic" changes have traditionally not been associated with IBS. IBD is usually classified as ulcerative colitis or Crohn disease, but it also includes forms of microscopic colitis, *eg*, histologic evidence of mucosal inflammation without macroscopic abnormalities. IBD is characterized by a constellation of patient-reported history and endoscopic, histopathologic, and radiologic findings, often with serologic correlates. Classic signs that reflect the inflammatory process within the gastrointestinal tract are rectal bleeding, diarrhea, fever, and weight loss, occasionally associated with extraintestinal manifestations. Interestingly, in the absence of complications, abdominal pain is not necessarily the most prominent symptom in IBD, despite extensive mucosal inflammation and presumably sensitization of peripheral visceral pain pathways. Genetic predisposition, environmental factors, infectious agents, altered gut epithelial permeability, and impaired immune responses have been incriminated in the still unclear cause of IBD.

By contrast, IBS, classified as functional (as opposed to organic) bowel disorder, is currently diagnosed on the basis of a characteristic cluster of symptoms in the absence of detectable structural abnormalities. As a matter of fact, according to the currently used symptom criteria (Rome criteria), once organic changes are detected, a diagnosis of a functional syndrome can no longer be made [1]. Because of the nonspecificity of the cardinal symptoms of abdominal pain or abdominal discomfort (the latter including bloating-type symptoms, a sensation of rectal urgency, or incomplete evacuation), the current diagnosis of IBS applies to a heterogeneous group of patients, even after attempts to define subgroups based on predominant bowel habit. Current theories to explain the pathophysiology of IBS include alteration in visceral perception, gastrointestinal motility and gut epithelial and immune function. Considerable evidence supports a role of psychosocial and physical (*ie*, gastroenteric infections) stressors as central and peripheral triggers, respectively, of first symptom onset or exacerbation [2•]. As reflected by an increasing number of publications on the subject, considerable interest in the putative role of low-

grade chronic inflammation in the pathogenesis of IBS has recently emerged [3]. Enhanced responsiveness to psychosocial and physical stressors has been suggested as a plausible mechanism that could explain most clinical and experimental findings in IBS, and that is consistent with the majority of the reported physiologic alterations [4].

### Evidence of mucosal immune activation in patients meeting symptom criteria for inflammatory bowel diseases

Several recent independent studies have demonstrated alterations in the gut-associated immune system. Quantitative assessment in unselected patients with IBS have shown increased mast cell numbers in the ileum [5] and colonic mucosa [6]. Preliminary evidence suggests an increase of overall cellularity in the colonic mucosa [7] and a higher number of mast cells containing tryptase (known to have proinflammatory effects) in the colonic lamina propria of patients with IBS [8]. Additional preliminary results indicate a significant increase of inducible nitric oxide synthase (iNOS) expression in the colonic mucosa from unselected patients with IBS compared with control patients [9]. In the human colon, upregulation of iNOS has been implicated in inflammatory processes, and increased expression has been documented in IBD [10]. More recently, a study by Chadwick *et al.* [11•] demonstrated intestinal mucosal immune activation in 77 symptomatic patients meeting the Rome criteria (the authors did not specify Rome I vs II criteria). The study included patients with diarrhea, constipation, or both. In 38 of the patients (50%), a normal conventional histologic appearance was seen, but the immunohistologic results were abnormal (intraepithelial lymphocytes-IEL, lamina propria CD25+ and CD3+ lymphocytes). In 40% of patients, nonspecific microscopic inflammation was seen, whereas immunohistologic results showed similar increases in lymphocyte populations as in the first group. However, in contrast to the first group, they also showed increased numbers of neutrophils and mast cells. Ten percent of patients fulfilled the histologic and immunohistologic criteria for lymphocytic colitis. Even though the magnitude of changes in cell numbers was far less than observed in patients with IBD, the increased numbers of IEL, T cells, IL-2 receptor expressing cells, suppressor/cytotoxic T cells, and NK cells were consistent with an increased inflammatory cell presence in a subset of patients with altered bowel habits who met the symptom-based Rome criteria. Because a significant number of patients meeting the Rome criteria also met the histologic criteria for a diagnosis of lymphocytic colitis, the findings highlight a major problem with the way we currently diagnose IBS. By definition, the diagnosis of an organic disease such as lymphocytic colitis is inconsistent with a diagnosis of IBS. Furthermore, it is unclear whether the patients met the Rome criteria because of the presence of discomfort (urgency, bloating) relieved by bowel movements, or whether they re-

ported abdominal pain. Using the current Rome criteria, a diagnosis of IBS can be made in any patient experiencing abdominal discomfort (for example, in the form of urgency or bloating-type symptoms), that is relieved by a bowel movement. In the absence of mucosal histology to rule out macroscopic or microscopic forms of colitis, such a symptom cluster is likely to include a wide range of syndromes with different causes and pathologic mechanisms.

Another study reported neuromuscular and inflammatory abnormalities in the small bowel of 10 patients (8 women; age range 24–55 years) with severe IBS symptoms [12]. Surprising for an IBS population, the symptoms apparently were severe and refractory enough to justify a laparoscopic full-thickness biopsy. The durations of IBS symptoms ranged from 2 to 30 years, and the predominant bowel habits included constipation, diarrhea, and alternating bowel habits. In this study, analysis of full-thickness biopsy specimens of the jejunum from IBS patients (diagnosis having been made on the basis of absence of detectable structural lesions and fulfillment of the Rome I criteria for IBS) showed several histopathologic abnormalities. The authors reported in most patients some neural degeneration in the ganglia of the myenteric plexus associated with infiltration of CD3+ T lymphocytes and longitudinal muscle hypertrophy. In some cases, IEL numbers were increased, and the numbers of interstitial cells of Cajal were also increased. There are two major problems with the reported findings. First is the absence of an appropriate control group. For example, the observed mucosal alterations in the *proximal jejunum* were compared with biopsy specimens obtained from the *distal ileum* during colonoscopy, and alterations in the jejunal wall were compared with findings obtained in tissues from deceased patients (of unspecified sex and age). Second, as admitted by the authors, the patients in this study represented a highly selected group with severe symptoms that were apparently refractory to current management. Even though it was stated that patients had normal or nonspecific changes on small intestinal manometry, it is conceivable that the patients had a mild or early form of chronic intestinal pseudoobstruction. Analogous to the comments made above about the nonspecificity of the Rome criteria to differentiate microscopic colitis from IBS, the same argument could be made for chronic intestinal pseudoobstruction.

Patients in another group, frequently discussed as evidence for a possible role of altered gut immune function in IBS, are those in whom IBS-like symptoms develop after a documented gastroenteric infection (so-called postinfectious IBS [PI-IBS] patients). A history of acute gastroenteritis caused by a variety of bacterial infections as well as parasitic infections was found to increase the risk of the development of persistent IBS symptoms.



The risk factors associated with PI-IBS include female gender, duration of the acute illness episode, and a major stressful life event at the time of the infection. Patients with PI-IBS have been reported to show changes in gut motility (eg, reduced rectal compliance) and epithelial function and an increase in enterochromaffin cells [13,14]. In addition, mucosal immune parameters in these patients exhibit changes that include altered macrophage (CD68) and T lymphocyte (CD3, CD4, CD8) populations and increased expression of IL-1 $\beta$  mRNA [15]. Some of these changes, as well as symptoms of diarrhea, were shown to persist for more than a year in some cases, suggesting chronic immune system activation [15]. Although the mechanisms involved in the ongoing inflammation after clearance of the infectious agent remain unclear, it has been suggested that a subset of IBS patients may have a genetic predisposition to inflammatory dysregulation. Preliminary evidence suggests a reduced frequency of the high producer allele for the antiinflammatory cytokines IL-10 and TGF- $\beta$ , suggesting a reduced production of these cytokines in patients with IBS compared with healthy control subjects [16]. Several important questions have to be addressed before the existence of a distinct pathophysiologic entity of PI-IBS can be confirmed. (1) Even though persistence of low-grade inflammation has been described in individuals who continued to be symptomatic, a causal role of these mucosal changes with IBS symptoms has not been demonstrated [14,15]. Preliminary reports from a therapeutic trial with an antiinflammatory agent in PI-IBS did not demonstrate any effect on symptoms [17]. (2) There is currently no evidence of visceral hypersensitivity in this patient group, and the reported lower volume thresholds for discomfort simply reflect a reduced rectal compliance. (3) It is unclear whether patients who report their first onset of IBS symptoms after an enteric infection have a history of other intestinal or extraintestinal functional syndromes (such as dyspepsia or chronic constipation) or anxiety disorders. In this case, the persistence of bowel symptoms may simply be a reactivation of a preexisting functional syndrome.

Tibble *et al.* [18••] compared a large population of patients with altered bowel habits meeting the Rome I criteria for IBS and patients with different organic diseases of the intestine, including IBD, cancer, infectious diarrhea, and celiac disease. They observed that markers for intestinal inflammation, such as fecal calprotectin levels, were elevated in the majority of patients with organic gastrointestinal conditions and decreased in the majority of patients with IBS. The sensitivity and specificity of fecal calprotectin levels for organic intestinal disease were 89% and 79%, respectively. However, the authors observed a significant number of IBS patients whose fecal calprotectin levels were above a normal cutoff value, suggesting some degree of inflammation.

Taken together, the above findings are most consistent with the concept that in a subset of patients meeting the current diagnostic criteria for IBS, chronic low-grade immune activation may be associated with chronic changes in gut motor and secretory function resulting in chronic abdominal discomfort associated with altered bowel habits. However, a causal relationship between visceral hypersensitivity and chronic immune activation has not been demonstrated.

### Altered immune system and inflammation in inflammatory bowel diseases

Classic histopathologic inspection of tissue from patients with IBD reveals vasodilatation, venocongestion, edema, infiltration of large numbers of inflammatory cells (lymphocytes as well as macrophages and monocytes), and architectural disarray, often with mucosal erosions and/or frank ulcerations. Although the causative triggers remain unclear, the role of a persistent and likely dysregulated mucosal immune response is central to the pathogenesis of IBD. However, it remains unclear whether the persistent inflammation, an intrinsic feature of IBD, reflects a primary aberration in mucosal response or results from an inappropriate persistent stimulation. Accumulating evidence indicates that excessive activation of immunoinflammatory responses in IBD may be initiated by luminal flora. In this regard, recent data showed no difference in the overall composition of mucosal flora in patients with IBD and control subjects but demonstrated a higher concentration of mucosa-associated bacteria in patients with IBD [19]. The authors suggest that the changes in the concentrations of mucosal flora in IBD are not secondary to inflammation but result from a host-specific altered immunoinflammatory mucosal response to "self-flora" in susceptible individuals. The role of genetic factors continues to be explored, with disease susceptibility associated with genetic markers for particular subsets of IBD patients. Recent studies using genome-wide screening provided the first link between NOD2 mutations and the clinical characterization of Crohn disease [20,21]. NOD proteins are thought to be cytosolic receptors for bacterial signals, and NOD2 is expressed in monocytes and activates nuclear factor  $\kappa$ B (NF- $\kappa$ B). However, the mechanisms by which NOD2 mutations contribute to Crohn disease need further investigation. It has been hypothesized that different concentrations of bacteria in the ileum relative to the colon may contribute to the association between NOD2 mutations and ileal disease. A genetic background was also identified in ulcerative colitis associated with HLA genes and regions of the chromosomes 3, 7, and 12 [22]. In a recent review, Ardizzone *et al.* [23••] compiled the genetic factors recently involved in the pathogenesis of IBD. Considering the central role of cytokines in modulating intestinal inflammation, several studies have focused on cytokine genes, looking for mutations or polymorphisms and expression dysregulation [24]. In Crohn disease, an in-

creased expression of T-helper-1 (Th1) cytokines was initially described, whereas an atypical Th2 response was associated with ulcerative colitis, but this assessment is now thought to be too simplistic. Cytokine gene-regulated differences between and within the diseases are clearly more complex. Advances in the understanding of the immune response in IBD have stimulated the development of new therapeutic agents directed against key players in the inflammatory process. A range of therapeutic strategies to block the biosynthesis or action of proinflammatory cytokines, acting directly or through targeting immunoregulatory cytokines, has been developed [25].

Among specific targets, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) was among the first mucosal cytokines identified as critical in the development and amplification of mucosal inflammation in IBD [26]. Recent clinical trials showed that anti-TNF- $\alpha$  antibodies provide marked clinical benefits in some patients with Crohn disease: a translational insight that has now become commonplace in IBD clinical therapy [27,28]. An inhibitor of mitogen-activated protein kinase (MAPK) appears to be another candidate in novel therapeutic strategies. A beneficial effect of CNI-1493 (MAPK inhibitor) in patients with severe Crohn disease was recently described [29]. A better characterization of the molecular signaling pathways involved in the activation of key immune and inflammatory cells will indubitably provide new targets for the development of therapeutic agents for IBD.

### **What unifies and separates irritable bowel syndrome and inflammatory bowel diseases**

#### **Possible role of failure to downregulate**

##### **Immune response**

A comparison of published data on the activation of the gut-associated mucosal immune system in IBS and IBD reflects both the similarities and the differences in the altered immune response observed in these disorders. However, the triggering factor initiating the inflammatory response remains unclear. In IBS, an immune response to infection [30], a disinhibition of the immune system during chronic sustained stress (Fig. 1), or a combination of both are plausible mechanisms that could result in the initial immune activation. The persistence of low-grade inflammation after pathogen clearance or after resolution of the psychosocial stressor, in a subset of individuals, may be related to an inability to efficiently downregulate the inflammatory response. This inability may be related to genetic factors or to early programming of antiinflammatory systems, such as the hypothalamic-pituitary-adrenal (HPA) [31•]. For example, a hyporesponsive HPA axis in the Lewis rat has been shown to be associated with exaggerated immune responses to various stimuli, including chemically induced colitis [32]. The most recent available data on IBD increasingly em-

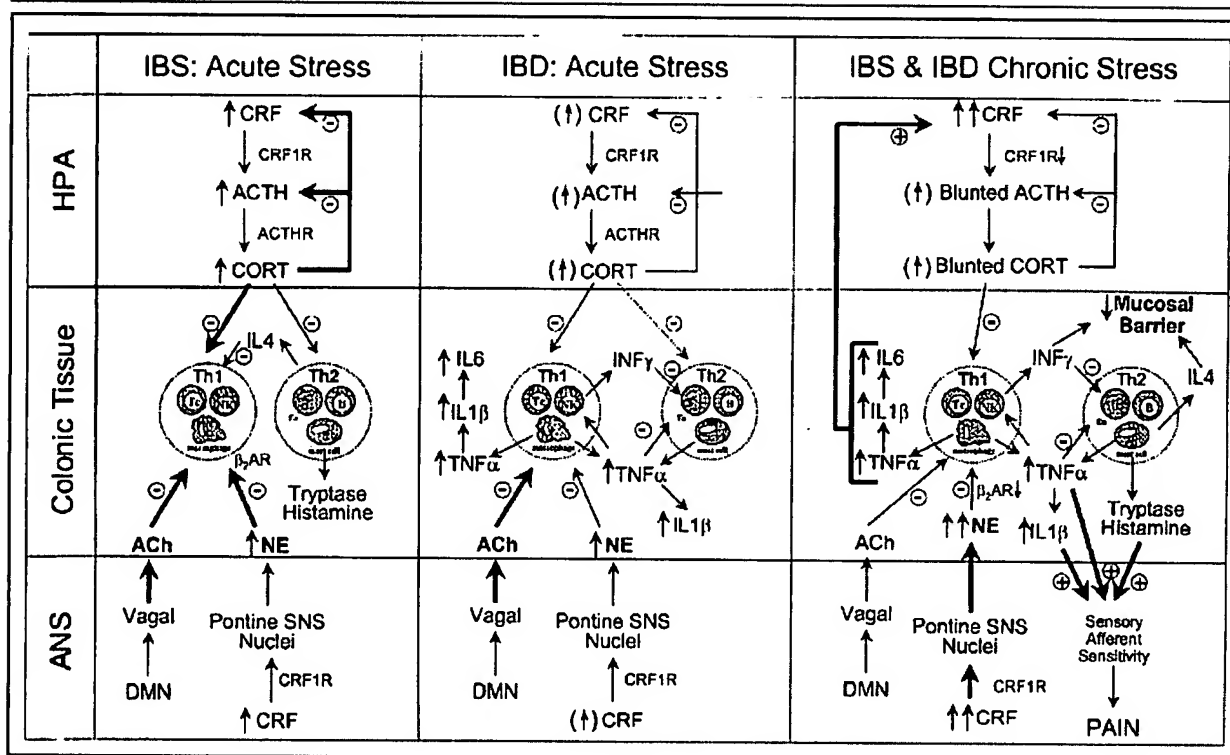
phasize the role of immunogenetics in the predisposition, modulation, and perpetuation of the disease [33]. The abnormal amplification and persistence of inflammation leading to tissue injuries likely reflects the continuing presence of the driving stimulus and self-reinforcing activation of mucosal inflammatory cells mediated by increased expression of cytokines.

##### **Increased permeability**

For both syndromes, histologic and functional alterations of the mucosal barrier have been recently reported [11•,12,24,25]. Small intestinal permeability is abnormal in a wide variety of conditions affecting the small intestine, including celiac disease, Crohn disease, and intestinal infections [18••]. Interestingly, gut permeability assessed by the lactulose/mannitol ratio is significantly elevated in PI-IBS patients [15]. This functional alteration of the intestinal barrier function may be a cause or consequence of inflammation, and a direct link between increased intestinal permeability and the exaggerated immune activation in IBD still needs to be confirmed. In addition to a causative role of peripheral factors, gut permeability changes in animal models have also been reported in response to various stressors. For example, in a rat model of chronic stress, an increase in intestinal epithelial permeability, associated with an increase in mucosal neutrophils and mast cells, has been demonstrated [34••]. In this model, the combination of stress-induced increases in intestinal permeability, allowing easier access of antigens to gut-associated macrophages and dendritic cells, together with stress-induced changes in HPA axis responsiveness and cytokine profiles, resulted in the development of colitis, without any additional chemical or immunologic manipulations. Rats with a history of aversive early life events were more susceptible to these stress-induced changes in gut permeability [35•], possibly related to early programming of the HPA axis [31•].

##### **Changes in luminal flora**

A change in intestinal microflora has been implicated, in association with genetic factors, as a putative mechanism responsible for the initiation and persistence of inflammation in IBD. Indeed, it has been suggested that the failure to maintain immunologic tolerance toward the indigenous microflora leads to a disease-associated dysregulation of the gut-associated immune system. Direct and indirect evidence of altered flora of the large and small intestine has been reported in IBS patients. For example, Balsari *et al.* [36] observed a decrease in coliforms, lactobacilli, and, to some extent, bifidobacteria in a small group of IBS patients. More recently, preliminary evidence of an alteration of bacterial concentration in colonic biopsy specimens from IBS patients has been reported [37]. Indirect evidence for bacterial overgrowth of the small intestine (in the form of altered hydrogen breath test results) has been reported in patients with IBS, and a recent randomized controlled trial found evi-

**Figure 1. Brain-gut immune interactions in irritable bowel syndrome and inflammatory bowel disease: effect of chronic stress on the mucosal immune system**

Acute stress causes increases in the activity of the hypothalamic-pituitary-adrenal (HPA) axis and of the two branches of the autonomic nervous system (ANS), the sympathetic nervous system (SNS), and the parasympathetic (vagal) system. In patients with irritable bowel syndrome, the peripherally acting products of each of these pathways (cortisol, CORT; norepinephrine, NE; acetylcholine, ACh) can inhibit the mucosal immune system, especially Th1-type responses. This results in a temporary shift toward Th2 cytokine responses (IL-4 and others) that are not as strongly inhibited and that can further inhibit Th1 responses. In patients with inflammatory bowel diseases, the corticotropin-releasing factor (CRF) response may be blunted, leading to diminished CORT and NE release. These changes favor the production of Th1 cytokines and the proliferation of macrophages, natural killer (NK) cells, and cytotoxic T cells (Tc). TNF $\alpha$ , which is produced by activated macrophages but can also be released by activated mast cells, stimulates the production of IL-1 $\beta$  (in the Th1 pathway) and IL-6 (by lymphoid and nonlymphoid tissues). With chronic stress in both types of patients, the shift to a Th1 response becomes predominant, with positive feedback loops developing between the gut and the brain. The restraints on immune cell proliferation and activation are compromised by blunting of the HPA axis response due to downregulation of pituitary CRF1 receptors, decreased vagal tone, and downregulation of  $\beta_2$ -adrenergic receptors ( $\beta_2$ -AR) on Th1 immune cells by chronically elevated catecholamines. Circulating levels of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 increase to concentrations that synergistically stimulate CRF production in the PVN of the hypothalamus. In irritable bowel syndrome, TNF $\alpha$  and IL-1 $\beta$  sensitize primary afferent terminals through long-lasting effects on gene expression, including the expression of neurokinin receptors. Locally acting mast cell products (tryptase and histamine) and proinflammatory compounds (PGE $_2$ ) can also sensitize primary afferents. Both IFN $\gamma$  (Th1 cytokine), which is produced by NK cells in response to TNF $\alpha$ , and IL-4 (a Th2 cytokine) have been shown to decrease mucosal barrier function by increasing epithelial permeability [54,55], thus perpetuating a local inflammatory response by allowing entry of bacteria and bacterial products. Subjective pain responses to peripheral sensitization of visceral afferents in irritable bowel syndrome and inflammatory bowel diseases are likely to be modulated differentially by endogenous pain modulation pathways. DMN, dorsal motor nucleus of the vagus; ACTH, adrenocorticotropic hormone.

dence that antibiotic treatment was beneficial for IBS symptoms of bloating and discomfort [38]. Based on the concept of altered interactions between the colonic flora and the gut-associated immune system, probiotics have been proposed as an alternative strategy for the treatment of several gastrointestinal diseases, including IBD [39] and more recently IBS [40,41]. However, the reported results are conflicting, and only a small number of double-blind controlled clinical trials support a beneficial health effect in IBD or IBS [42]. The epithelium has recently been recognized as playing an important role in innate immune responses in response to intestinal microorganisms [43,44]. It expresses a variety of receptors (Toll-like receptor) involved in the recognition of a spectrum of microbial products. This recognition capability

may enable an appropriate cytokine and chemokine secretion in response to changes in luminal flora.

#### **Influence of sustained psychosocial stressors on mucosal immune system activation**

Even though stress has been less recognized as a factor in the natural history of IBD, considerable evidence supports a prominent role for it in the pathophysiology and clinical presentation of both IBD and IBS symptoms [45]. Patients with IBS seem to have a greater reactivity to stress than do control subjects or IBD patients. Yet, sustained psychologic stressors have been associated with the onset and exacerbation of symptoms in both IBS and IBD [46–48]. The development of persistent IBS symptoms after acute gastroenteritis has been asso-

ciated with major life events around the time of infection [14]. Similarly, for IBD, a wide range of clinical studies indicates a strong link between sustained psychosocial stressors and IBD activity [49]. Levels of long-term perceived stress have been shown to correlate with changes in mucosal appearance and relapse in ulcerative colitis [50•]. Further evidence of an influence of stress on inflammatory processes comes from animal studies showing a modulation of the immune function at different levels, including immune cell distribution, cytokine profiles, or susceptibility to infection in naïve or colitic animals [51]. In view of the established concept of an altered immune response in IBD patients, and the suspected low-grade inflammation in some patients meeting the symptom criteria for IBS, it is reasonable to consider a bidirectional model of brain-gut interactions as an important determinant of gut-associated immune activation in both disorders.

#### Chronic Inflammation and alteration of sensory-motor functions of the gastrointestinal tract

Despite the common assumption that chronic gut mucosal inflammation is associated with sensory-motor dysfunction of the gastrointestinal tract in inflammatory as well as functional intestinal disorders, the relationship between *chronic* inflammation and the generation of gastrointestinal symptoms remains unclear. The development of IBS-like symptoms in some patients with quiescent ulcerative colitis was suggested as an indication of the role of inflammation on altered sensory and motor function [8]. The concept of long-lasting postinflammatory changes in gut motility is supported by the observation of altered anorectal and colonic motility in patients in remission from ulcerative colitis and Crohn disease [52]. However, chronic abdominal pain and visceral hypersensitivity—classic features in patients with IBS—do not appear to be a hallmark of ulcerative colitis or Crohn disease [53]. One may speculate that various patient populations with different degrees of intestinal inflammation (patients with IBD and PI-IBS, and possibly small subsets of those with IBS) do not necessarily experience pain and discomfort from these mucosal changes. Whereas the effects of the immune activation are likely to affect enteric nervous system circuits and smooth muscle function, altering intestinal compliance and reflex activity and producing such symptoms as diarrhea and urgency, the effects on visceral perception are less predictable. An important variable in symptom generation is the differences in the ability of the brain and its endogenous pain inhibitory pathways to counteract the changes in peripheral viscerosensory pathways.

#### Conclusion

The recent observation of an activated immune system in some IBS patients associated with persistent low-grade mucosal inflammation provides evidence for the reconsideration of the symptom-criteria-based diagnosis

of functional bowel disorders. The development and use of biologic markers identifying low-grade inflammation would improve the characterization of subsets of IBS patients in whom peripheral mechanisms may participate in specific symptom genesis and could be considered in the choice of the therapy.

#### References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- Of special interest
  - \*\* Of outstanding interest
- 1 Drossman DA, Corazziari E, Talley NJ, et al.: Rome II. The functional gastrointestinal disorders. Diagnosis, pathophysiology and treatment: a multinational consensus. 2<sup>nd</sup> ed. McLean, VA: Degnon Associates, 2000
  - 2 Mayer EA, Collins SM: Evolving pathophysiologic models of functional gastrointestinal disorders. *Gastroenterology* 2002, 122:2032–2048.  
Comprehensive review of evidence for peripheral and central mechanisms contributing to symptom generation in IBS.
  - 3 Spiller RC: Neuropathology of IBS? *Gastroenterology* 2002, 123:2144–2147.
  - 4 Mayer EA, Naliboff BD, Chang L, et al.: V. Stress and irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol* 2001, 280:G519–G524.
  - 5 Weston AP, Biddle WL, Bhatia PS, et al.: Terminal ileal mucosal mast cells in irritable bowel syndrome. *Dig Dis Sci* 1993, 38:1590–1595.
  - 6 O'Sullivan M, Clayton N, Breslin NP, et al.: Increased mast cells in the irritable bowel syndrome. *Neurogastroenterol Motil* 2000, 12:449–457.
  - 7 Barbara G, Stanghellini V, De Giorgio R, et al.: Neuroimmune relationships in the colonic mucosa of irritable bowel syndrome patients. *Neurogastroenterol Motil* 2000, 12:A272.
  - 8 Barbara G, Cottrell G, Grady E, et al.: Expression and release of mast cell tryptase in irritable bowel syndrome (IBS). *Gastroenterology* 2002, 122:A276.
  - 9 O'Sullivan MA, Clayton N, Wong T: Increased inos and nitrotyrosine expression in irritable bowel syndrome. *Gastroenterology* 2000, 118:A702.
  - 10 Miller MJ, Sandoval M: Nitric oxide: III. A molecular prelude to intestinal inflammation. *Am J Physiol Gastrointest Liver Physiol* 1999, 276:G795–G799.
  - 11 Chadwick VS, Chen W, Shu D, et al.: Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* 2002, 122:1778–1783.  
Interesting paper showing evidence for mucosal immune activation in patients who also meet the current diagnostic criteria for IBS.
  - 12 Tornblom H, Lindberg G, Nyberg B, et al.: Full-thickness biopsy of the jejunum reveals inflammation and enteric neuropathy in irritable bowel syndrome. *Gastroenterology* 2002, 123:1972–1979.
  - 13 Gwee KA, Collins SM, Marshall JS, et al.: Evidence of inflammatory pathogenesis in post-infectious irritable bowel syndrome. *Gastroenterology* 1998, 114:A758.
  - 14 Gwee KA, Leong YL, Graham C, et al.: The role of psychological and biological factors in postinfective gut dysfunction. *Gut* 1999, 44:400–406.
  - 15 Spiller RC, Jenkins D, Thornley JP, et al.: Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute *Campylobacter* enteritis and in post-dysenteric irritable bowel syndrome. *Gut* 2000, 47:804–811.
  - 16 Chan J, Gonsalkorale WM, Perrey C, et al.: IL-10 and TGF- $\beta$  genotypes in irritable bowel syndrome: evidence to support an inflammatory component? *Gastroenterology* 2000, 118:A184.
  - 17 Dunlop S, Jenkins D, Naesdal J, et al.: Randomised double-blind placebo-controlled trial of prednisolone in post-infectious irritable bowel syndrome. *Gastroenterology* 2002, 122:A60.
  - 18 Tibble JA, Sigthorsson G, Foster R, et al.: Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. *Gastroenterology* 2002, 123:450–460.  
Large clinical study comparing biologic markers that may be useful to differentiate inflammatory from functional disorders of the gut. The results suggest that mucosal inflammation and increased small intestinal permeability are not found in the majority of IBS patients.

- 19 Swidsinski A, Ladhoff A, Pernthaler A, et al.: Mucosal flora in inflammatory bowel disease. *Gastroenterology* 2002, 122:44–54.
- 20 Ahmad T, Amuzzi A, Bunce M, et al.: The molecular classification of the clinical manifestations of Crohn's disease. *Gastroenterology* 2002, 122:854–868.
- 21 Cuthbert AP, Fisher SA, Mirza MM, et al.: The contribution of NOD2 gene mutations to the risk and site of disease in inflammatory bowel disease. *Gastroenterology* 2002, 122:867–874.
- 22 Satsangi J, Parkes M, Louis E, et al.: Two-stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet* 1996, 14:199–202.
- 23 Ardizzone S, Porro GB: Inflammatory bowel disease: new insights into pathogenesis and treatment. *J Intern Med* 2002, 252:475–496.  
Comprehensive and extensive review of the mechanisms associated with the development of IBD and the new therapeutic approaches in IBD.
- 24 Laroux FS, Grisham MB: Immunological basis of inflammatory bowel disease: role of the microcirculation. *Microcirculation* 2001, 8:283–301.
- 25 Van Montfrans C, Peppelenbosch M, Te Velde AA, et al.: Inflammatory signal transduction in Crohn's disease and novel therapeutic approaches. *Biochem Pharmacol* 2002, 64:789–795.
- 26 Kollias G, Kontoyiannis D: Role of TNF/TNFR in autoimmunity: specific TNF receptor blockade may be advantageous to anti-TNF treatments. *Cytokine Growth Factor Rev* 2002, 13:315–321.
- 27 Hommes DW, van de Heisteg BH, van der Spek M, et al.: Infliximab treatment for Crohn's disease: one-year experience in a Dutch academic hospital. *Inflamm Bowel Dis* 2002, 8:81–86.
- 28 Ardizzone S, Colombo E, Maconi G, et al.: Infliximab in treatment of Crohn's disease: the Milan experience. *Dig Liver Dis* 2002, 34:411–418.
- 29 Hommes DW, Peppelenbosch MP, van Deventer SJ: Mitogen activated protein (MAP) kinase signal transduction pathways and novel anti-inflammatory targets. *Gut* 2003, 52:144–151.
- 30 Collins SM, Piche T, Rampal P: The putative role of inflammation in the irritable bowel syndrome. *Gut* 2001, 49:743–745.
- 31 Matthews SG: Early programming of the hypothalamo-pituitary-adrenal axis. *Trends Endocrinol Metab* 2002, 13:373–380.  
Excellent summary of evidence supporting the concept that prenatal aversive events are able to permanently alter the responsiveness of the HPA axis in the offspring.
- 32 Million M, Tache Y, Anton P: Susceptibility of Lewis and Fischer rats to stress-induced worsening of TNB-colitis: protective role of brain CRF. *Am J Physiol Gastrointest Liver Physiol* 1999, 276:G1027–G1036.
- 33 Podolsky DK: The current future understanding of inflammatory bowel disease. *Best Pract Res Clin Gastroenterol* 2002, 16:933–943.
- 34 Soderholm JD, Yang PC, Ceponis P, et al.: Chronic stress induces mast cell-dependent bacterial adherence and initiates mucosal inflammation in rat intestine. *Gastroenterology* 2002, 123:1099–1108.  
First report demonstrating that chronic stress without additional peripheral insults to the gut can result in the development of colitis in the rat. The involved mechanisms include a stress-induced increase in intestinal permeability.
- 35 Soderholm JD, Yates DA, Gareau MG, et al.: Neonatal maternal separation predisposes adult rats to colonic barrier dysfunction in response to mild stress. *Am J Physiol Gastrointest Liver Physiol* 2002, 283:G1257–G1263.  
Interesting study showing that early life stress can program an organism to show altered stress responses as an adult. The reported greater propensity of affected animals to the development of increased intestinal permeability during stress, together with previous reports on altered motility and perceptual responses to stress, emphasize the importance of early life events in programming the stress responsiveness of the adult animal.
- 36 Balsari A, Ceccarelli A, Dubini F, et al.: The fecal microbial population in the irritable bowel syndrome. *Microbiologica* 1982, 5:185–194.
- 37 Swidsinski A, Khilkin M, Ortner M, et al.: Alteration of bacterial concentration in colonic biopsies from patients with irritable bowel syndrome (IBS). *Gastroenterology* 1999, 116:A1.
- 38 Pimentel M, Chow EJ, Lin HC: Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome: a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol* 2003, 98:412–419.
- 39 Gionchetti P, Rizzello F, Venturi A, et al.: Probiotics in infective diarrhoea and inflammatory bowel diseases. *J Gastroenterol Hepatol* 2000, 15:489–493.
- 40 Sen S, Mullan MM, Parker TJ, et al.: Effect of *Lactobacillus plantarum* 299v on colonic fermentation and symptoms of irritable bowel syndrome. *Dig Dis Sci* 2002, 47:2615–2620.
- 41 Madden JA, Hunter JO: A review of the role of the gut microflora in irritable bowel syndrome and the effects of probiotics. *Br J Nutr* 2002, 88(Suppl 1):S67–S72.
- 42 Marteau PR, de Vrese M, Cellier CJ, et al.: Protection from gastrointestinal diseases with the use of probiotics. *Am J Clin Nutr* 2001, 73(Suppl 2):S430–S436.
- 43 Cario E, Podolsky DK: Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect Immun* 2000, 68:7010–7017.
- 44 Cario E, Rosenberg IM, Brandwein SL, et al.: Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. *J Immunol* 2000, 164:966–972.
- 45 Mayer EA: The neurobiology of stress and gastrointestinal disease. *Gut* 2000, 47:861–869.
- 46 Monnikes H, Tebbe JJ, Hildebrandt M, et al.: Role of stress in functional gastrointestinal disorders: evidence for stress-induced alterations in gastrointestinal motility and sensitivity. *Dig Dis* 2001, 19:201–211.
- 47 Levy RL, Cain KC, Jarrett M, et al.: The relationship between daily life stress and gastrointestinal symptoms in women with irritable bowel syndrome. *J Behav Med* 1997, 20:177–193.
- 48 LeResche L, Dworkin SF: The role of stress in inflammatory disease, including periodontal disease: review of concepts and current findings. *Periodontol* 2000 2002, 30:91–103.
- 49 Levenstein S, Prantera C, Varvo V, et al.: Stress and exacerbation in ulcerative colitis: a prospective study of patients enrolled in remission. *Am J Gastroenterol* 2000, 95:1213–1220.
- 50 Hart A, Kamm MA: Mechanisms of initiation and perpetuation of gut inflammation by stress. *Aliment Pharmacol Ther* 2002, 16:2017–2028.  
Comprehensive review of the mechanisms by which stress can alter intestinal physiologic functions. Identification of the different mediators involved in the brain-gut-immune axis contributing to the initiation, perpetuation, and reactivation of gut inflammation.
- 51 Collins SM, McHugh K, Jacobson K, et al.: Previous inflammation alters the response of the rat colon to stress. *Gastroenterology* 1996, 111:1509–1515.
- 52 Annesse V, Bassotti G, Napolitano G, et al.: Gastrointestinal motility disorders in patients with inactive Crohn's disease. *Scand J Gastroenterol* 1997, 32:1107–1117.
- 53 Chang L, Munakata J, Mayer EA, et al.: Perceptual responses in patients with inflammatory and functional bowel disease. *Gut* 2000, 47:497–505.
- 54 Madara JL, Stafford J: Interferon-gamma directly affects barrier function of cultured intestinal epithelial monolayers. *J Clin Invest* 1989, 83:724–727.
- 55 Colgan SP, Resnick MB, Parkos CA, et al.: IL-4 directly modulates function of a model human intestinal epithelium. *J Immunol* 1994, 153:2122–2129.